Multiple factors contribute to outcrossing in a tropical emergent Dipterocarpus tempehes, including a new pollen-tube guidance mechanism for self-incompatibility

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Abstract: The self-rejection system of Dipterocarpus tempehes (Dipterocarpaceae), an emergent tree of the lowland tropical forests of Borneo, were studied by means of pollination experiments, fluorescence microscopy of pollen tubes, and monitoring of ovary maturation patterns. Fruit set was higher in cross-pollinated flowers than in control and self-pollinated flowers, indicating the existence of pollen limitation and a self-rejection system. Although the adhesion and the germination of self-pollen and the growth of self-pollen tubes were not inhibited, the proportion of cross-pollen tubes that entered the style was 1.7–2.3 times higher than that of self-pollen tubes, indicating a partial self-incompatibility that inhibits self-pollen tubes from entering the style hollow. These results suggest, for the first time, that self-incompatibility is caused by a defect of pollen-tube guidance. We also suggest a threshold effect in number of pollen tubes or late-acting self-incompatibility to fully explain the drastic and uniform selection against self-pollinated flowers before ovary swelling. After that, maternal selection and/or inbreeding depression caused the abortion and delayed maturation of self-pollinated flowers. The advantages of the self-rejection process composed of partial early-acting self-incompatibility and relatively strong delayed abortion is discussed with respect to the general-flowering phenomenon in lowland dipterocarp forests.

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MULTIPLE FACTORS CONTRIBUTE TO OUTCROSSING IN A TROPICAL EMERGENT *DIPTEROCARPUS TEMPEHES*, INCLUDING A NEW POLLEN-TUBE GUIDANCE MECHANISM FOR SELF-INCOMPATIBILITY[1]

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The self-rejection system of *Dipterocarpus tempehes* (Dipterocarpaceae), an emergent tree of the lowland tropical forests of Borneo, were studied by means of pollination experiments, fluorescence microscopy of pollen tubes, and monitoring of ovary maturation patterns. Fruit set was higher in cross-pollinated flowers than in control and self-pollinated flowers, indicating the existence of pollen limitation and a self-rejection system. Although the adhesion and the germination of self-pollen and the growth of self-pollen tubes were not inhibited, the proportion of cross-pollen tubes that entered the style was 1.7–2.3 times higher than that of self-pollen tubes, indicating a partial self-incompatibility that inhibits self-pollen tubes from entering the style hollow. These results suggest, for the first time, that self-incompatibility is caused by a defect of pollen-tube guidance. We also suggest a threshold effect in number of pollen tubes or late-acting self-incompatibility to fully explain the drastic and uniform selection against self-pollinated flowers before ovary swelling. After that, maternal selection and/or inbreeding depression caused the abortion and delayed maturation of self-pollinated flowers. The advantages of the self-rejection process composed of partial early-acting self-incompatibility and relatively strong delayed abortion is discussed with respect to the general-flowering phenomenon in lowland dipterocarp forests.

Key words: breeding system; canopy access; Dipterocarpaceae; inbreeding depression; Lambir Hills National Park; pollination experiment; pollen-tube guidance; self-incompatibility.

Among angiosperms, hermaphroditic plants have evolved self-rejection mechanisms, such as self-incompatibility, to enhance outcrossing (de Nettancourt, 1977). de Nettancourt (1977) suggested that in most cases post-fertilization self-rejection does not contribute extensively to outcrossing and that the term ''self-incompatibility'' should be used only for the pre-fertilization mechanism. On the other hand, Seavey and Bawa (1986) pointed out that post-fertilization self-rejection are quite common and proposed the concept of late-acting self-incompatibility. The relative contribution of such self-rejection systems varies according to life form or phylogenetic background (e.g., Seavey and Bawa [1986] pointed out that late-acting self-incompatibility is characteristic of woody plants). In addition to these early- and late-acting types of self-incompatibility, early-acting inbreeding depression (Wiens, 1984; Wiens et al., 1989) and late-acting maternal selection (Bookman, 1984; Stephenson and Winsor, 1986; Griffin, Moran, and Fripp, 1987; Kenrick and Knox, 1989; Waser, 1993) may also increase realized outcrossing rates. Characterizing such phenomena is necessary to understand how outcrossing is maintained within a population or species.

Recent studies in species-rich tropical rainforests have reported the predominance of outcrossing in trees (Bawa, 1974; Hamrick and Murawski, 1990; Kitamura et al., 1994; Murawski and Bawa, 1994), although Fedorov (1966) and Van Steenis (1969) claimed that trees must be self-compatible and self-pollinated because of the spatial isolation of individuals. High outcrossing rate indicates that the self-rejection systems are common in tree species of the tropical rainforest.

Dipterocarpus tempehes is an emergent tree of lowland tropical rainforests in southeast Asia. The breeding system of dipterocarps in this region may be influenced by general flowering, a phenomenon unique to this region. General flowering refers to supra-annual and irregular mass flowering, which is followed by mast fruiting at the community level. General flowering occurs at intervals of 5 yr on average (Ashton, Givinish, and Appanah, 1988; Appanah, 1993). During the general flowering period, a large portion of species of Dipterocarpaceae, and many species of other families, bloom heavily while they hardly bloom at all at another period (Ashton, Givinish, and Appanah, 1988; Appanah, 1993; Sakai et al., 1999b). The population size of pollinators in the forest also fluctuates depending on the magnitude of general flowering (Nagamitsu, 1998; Itioka et al., in press). These large fluctuations in the flowering synchrony of plants and the population size of pollinators probably result in uncertain pollination efficiency and variable availability of pollen source. In response, flexibility in the self-rejection system, including partial self-incompatibility and delayed abortion, could have evolved to allow the proper choice of parents depending on the variable

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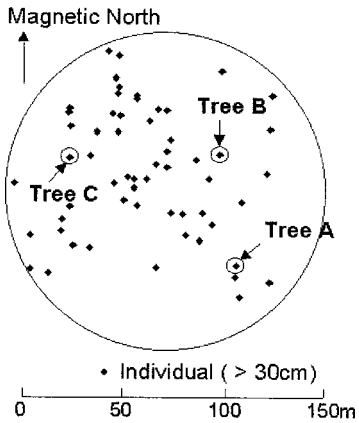


Fig. 1. Distribution of *Dipterocarpus tempehes* (diameter at breast height >30 cm) around Trees A, B, and C, which were investigated in this study. All mature trees inside the circle are indicated.

conditions. To date, however, studies on the breeding systems of dipterocarps (Chan, 1981; Dayanadan et al., 1990; Sakai et al., 1999a) have given little attention to the relative importance of different self-rejection systems. Although such studies have estimated the existence of self-incompatibility, they have not attempted to clarify the actual systems and timing of self-rejection.

In this study, we investigated the breeding system and the possible existence of a self-rejection system enhancing outcrossing in *D. tempehes*. We addressed three questions in particular. (1) Does *D. tempehes* have a self-rejection system? (2) If so, what is this mechanism? (3) Is there a flexible self-rejection system, such as partial self-incompatibility or delayed self-rejection? We also discussed the possible advantages of such a flexible mechanism in *D. tempehes* with respect to the general-flowering phenomenon in the lowland dipterocarp forest.

MATERIALS AND METHODS

Study site and plant—The study site was a primary lowland dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20′ N, 113°50′ E; altitude, 150–250 m). In August 1992, a Canopy Biology Plot (CBP) of 8 ha (200 × 400 m) was demarcated for long-term monitoring of plant phenology and plant–animal interactions.

Dipterocarpus tempehes belongs to the family Dipterocarpaceae, a family of dominant emergent trees in the lowland forest of West Malaysia (Ashton, 1982). *Dipterocarpus tempehes* frequently reaches a height of >50 m. It flowers at supra-annual and irregular intervals, but with relatively higher frequency than other typical general-flowering trees, which flower only in the general-flowering periods (T. Kenta et al., unpublished data). The flower longevity is ~1 d. The flowers are 3–5 cm long and 2–4 cm in diameter and have hollow-type styles (K. K. Shimizu, personal observation). Flowers of *D. tempehes* open sequentially from the inflorescence base towards the apex. An ovary contains six ovules, and usually only one ovule can mature to seed. The mature fruit is 2–4 cm in diameter. In most dipterocarp species, some or all of the five calyces enlarge as the fruits grow and develop into fruit wings, which are thought to enhance fruit dispersal. However, fruit wings are not developed in this species. Mature seeds germinate in a few days after dispersal.

The breeding system and flower demography of three *D. tempehes* individuals (Trees A, B, and C) in and around the 8-ha plot of Lambir Hills National Park were studied from May to September of 1998, when many species of Dipterocarpaceae and other families in this region flowered (general flowering) (Kato et al., 2000). The distribution of adult (diameter at breast height >30 cm) *D. tempehes* trees around these three individuals is shown in Fig. 1. We used the single-rope climbing technique (Perry, 1978) to access the forest canopy. Because access to flowers by the single-rope climbing technique is limited to those just around the main stem and most parts of the crown surface cannot be reached in the case of tropical emergent trees, we fixed additional 30–50 m ropes between >15 branches per canopy, and the observations were conducted along these ropes after accessing the crown.

Pollination manipulation—Four pollination treatments were carried out on each of the three individuals: (1) SELF, (2) CROSS, (3) BAG, and (4) CONTROL. In the former three treatments, flowers were bagged with nylon-fiber mesh (mesh size ~0.3 mm) before onset of flowering to exclude flower visitors. In the SELF treatment, the flower was hand-pollinated with pollen of the same flower. In the CROSS treatment, pollen of Tree A was applied to flowers on Tree B, pollen of Tree B to those on Tree C, and pollen of Tree C to those on Tree A. Pollen for the CROSS treatment was collected from flowers that opened on the same day as the treated flowers in the crown or on water-soaked branches sampled 1–3 d before the treatment (water-soaked branches naturally produce flowers in a few days after sampling). In the BAG treatment, bagged flowers were kept intact. Although the SELF, CROSS, and BAG treatments were carried out in the same bag, only a single treatment was conducted on any given individual in the course of a single day to avoid pollen contamination inside bags. In the CONTROL treatment, flowers were not bagged and were naturally pollinated.

We monitored a total of 3437 flowers on 101 branches (mean = 34 flowers per bag) for the SELF, CROSS, and BAG treatments. For the CONTROL treatment, 2830 flowers on 67 branches were monitored.

Fruit maturation and germination of mature seeds—We monitored flower abscission and ovary development in all the treatments. Ovary development was recorded using five stage categories: I, before swelling (~0.7 cm in diameter); II, after swelling and smaller than 1 cm in diameter; III, 1–2 cm; IV, 2–3 cm; and V, >3 cm. Seeds in stages IV and V have the ability to germinate (T. Kenta, unpublished data). The census was carried out every 1–3 d during the flowering period (1 May–10 June) and every 1–4 wk thereafter until fruit dispersal.

After seed maturation (stages IV and V), sound mature seeds fell from branches and were trapped inside of the bags in the CROSS, SELF, and BAG treatments. They germinated in a few days within the bags. We calculated the germination rate of these mature seeds trapped within the bags as an index of seed viability.

We compared ovary survivorship from stages I to IV, I to II, and II to IV between treatments by chi-square tests. Days required for fruit maturation to stages II, III, and IV (as an index of the speed of fruit maturation) and germination rate of mature seeds between treatments were also analyzed by unpaired Student's *t* tests and chi-square tests, respectively. However, because of the small sample size, we pooled the data of the three trees and pooled and conducted the SELF and BAG treatments together (= SELF + BAG treatment, as the data for self-pollinated ovaries) when calculating the days required for fruit maturation and the germination rate. Analyzing fruits from SELF and BAG together is appropriate because fruit in BAG treatments should have originated from self-pollination. It is unlikely that the fruits in BAG treatment came from apomixis, because we did not observe multiple-embryo seeds, which are associated with apomixis in all the species of Dipterocarpaceae studied so far (Kaur et al., 1986).

Pollen-tube growth—To examine self-incompatibility, we performed an additional pollination experiment in which we observed the germination and growth of pollen tubes. We cut off flowers from the trees and immediately hand-pollinated them with self- or cross-pollen in our laboratory (in the same manner as used for the SELF and CROSS treatments above, respectively). In total, 38 flowers were treated and fixed in FAA solution (formaldehyde, acetic acid, and 70% ethanol in the ratio 5:5:90 v/v/v) 6 h after hand pollination. We also fixed other sets of flowers at 2, 3, and 4 h after pollination manipulation, but the germination rates of pollen tubes were still low in the flowers

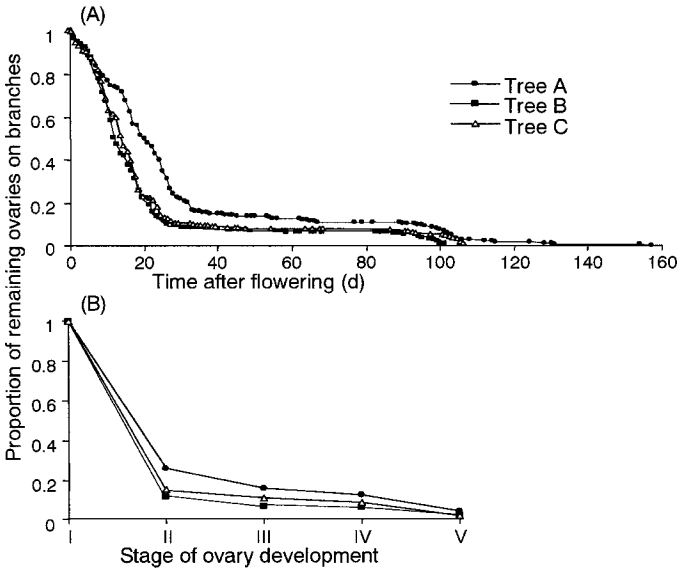


Fig. 2. Ovary survivorship curves in the CONTROL treatment for days after flowering (A) and stage of ovary development (B).

fixed earlier than 6 h. Then we analyzed only the results from flowers fixed at 6 h after manipulation.

After fixation of the flowers, pollen tubes were visualized following the method of Shimizu and Okada (2000) with some modifications. The fixed pistils were treated with 90%, 70%, and 40% ethanol for 20 min each in this order. After being washed with water, they were treated with 1 mol/L NaOH overnight. Then they were stained in 0.1% Aniline Blue (Schmid GMBH, Stuttgart, Baden-Württemberg, Germany) in 0.1% K_3PO_4 buffer (pH ~12.4) overnight. The pistils were mounted in glycerol and split into halves longitudinally using a 25-gauge needle. The state of pollen grains and the pattern of elongation of pollen tubes were observed under a Axiophoto2 (Carl Zeiss, Jena, Thuringia, Germany) microscope with UV illumination to visualize the callose of pollen tubes. The number of pollen tubes in the style was counted at a position 1 mm distant from the tip of the pistil. We also observed whether the fastest pollen tube reached the base of the style.

RESULTS

Survivorship and maturation pattern of ovaries—In the CONTROL treatment, a drastic decrease in the number of ovaries occurred in the first month after flowering. This was followed by a moderate decrease until fruit dispersal at 3–4 mo after flowering (Fig. 2A). The survivorship curve of ovaries along the developmental stages (Fig. 2B) shows that most ovaries abscised before stage II (the stage of ovary swelling), followed by a moderate decrease in the number of ovaries. The dissemination of mature fruits occurred after stage IV.

The survivorship curves of flowers in the CROSS, SELF, and BAG treatments were fundamentally similar to those in the CONTROL treatment: there was a rapid decrease in the number of ovaries before stage II and after stage IV (Fig. 3). Ovary survivorship from stages I to IV in CROSS treatments were 2.8–5.1 times higher than those in CONTROL treatments and 20–59 times higher than those in SELF treatments, and those in CONTROL treatments were 8–90 times higher than those in BAG treatments for all three individuals (Table 1). These differences were significant in all three trees (the order of the survivorship was CROSS > CONTROL > SELF = BAG, $P < 0.01$, chi-square test; Table 1). Very few fruits matured in the SELF and BAG treatments.

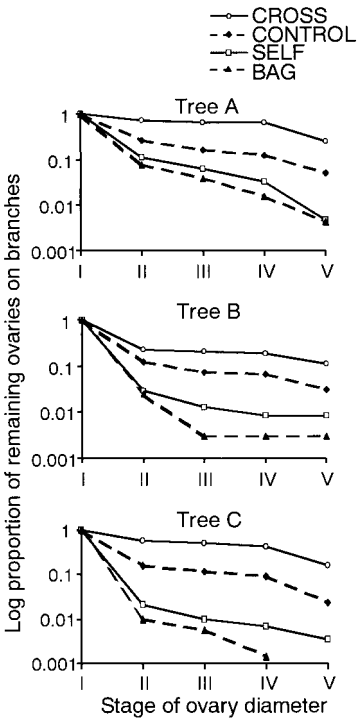


Fig. 3. Ovary survivorship curves in experimental treatments. The statistical results of differences between treatments are shown in Table 1.

Ovary survivorships from stages I to II in CROSS treatments were 6.2–29 times higher than those in SELF treatments, and the differences were significant in all three trees ($P < 0.01$, chi-square test; Table 1). Ovary survivorships from stage II to IV in CROSS treatments were 2.2–3.1 times higher than those in SELF treatments, and the differences were significant for Trees A and B ($P < 0.05$; Table 1) but not significant for Tree C ($P = 0.15$).

Fruit-maturation rate—In both treatments, fruits grew slowly before stage II (1 cm in ovary diameter) and faster at the later stages. Fruits matured more quickly in CROSS treatments (Fig. 4) than in SELF + BAG treatments. Significantly

TABLE 1. Ovary survivorships of states I–IV, I–II, and II–IV in each treatment in the three trees. Ovary survivorship from stages I to IV corresponds to fruit set. Values sharing the same letters were not significantly different from each other ($P < 0.05$, chi-square test). N = number of treated flowers.

Tree	Treatment	N	Survivorship between stages (%)		
			I–IV	I–II	II–IV
A	CROSS	45	64.4^{A}	71.1^{A}	90.6^{A}
	CONTROL	515	12.6^{B}	26.0^{B}	48.5^{B}
	SELF	210	3.3^{C}	11.4^{C}	29.2^{BC}
	BAG	699	1.6^{C}	7.7^{C}	20.4^{C}
B	CROSS	54	18.5^{A}	22.2^{A}	83.3^{A}
	CONTROL	478	6.5^{B}	11.9^{B}	54.4^{AC}
	SELF	240	0.8^{C}	2.9^{BC}	28.6^{C}
	BAG	662	0.3^{C}	2.4^{C}	12.5^{C}
C	CROSS	56	41.1^{A}	57.1^{A}	71.9^{A}
	CONTROL	92	9.8^{B}	12.0^{B}	81.8^{A}
	SELF	300	0.7^{C}	2.0^{C}	33.3^{AB}
	BAG	700	0.1^{C}	1.0^{C}	14.3^{BC}

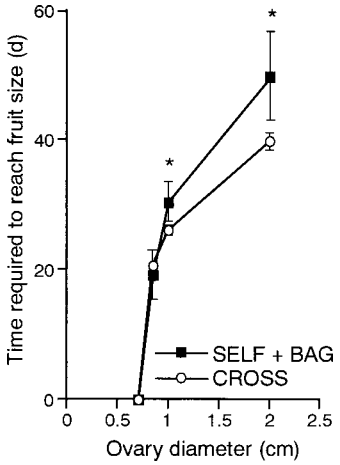


Fig. 4. Fruit maturation speed in CROSS and SELF + BAG (the data in SELF and BAG were treated together) treatments. The data from all three trees were pooled in both treatments. Ovary diameters of 0.7, 1.0, and 2.0 cm correspond to the average ovary diameters of stages I, III, and IV, respectively. Stage V was removed from this result due to the extremely small sample size. Fruits matured slowly until reaching 1.0 cm in size, at which point size increased precipitously. Flowers in the CROSS treatments took significantly fewer days (indicated by *, $P < 0.05$, unpaired Student's *t* test) to achieve 1.0- and 2.0-cm ovaries.

fewer days were required for maturation to 1 cm and 2 cm ovary diameter in CROSS treatments ($P < 0.05$, unpaired Student's *t* test). The difference in required days became greater at later stages.

Germination rate of mature seeds—The germination rate of mature seeds (= stage IV and V) was 1.6 times higher in CROSS than in SELF + BAG treatments (0.50 and 0.32, respectively), but the difference was not statistically significant ($P = 0.15$, chi-square test).

Pollen tube growth—The stigma (tip of the pistil) of *D. tempehes* was covered with stigmatic exudate (Figs. 7 and 10). In both the SELF and CROSS treatments, pollen grains were deposited on the stigmatic exudate as under natural conditions. The average numbers of pollen grains that germinated in the stigmatic exudate per pistil were 27.4 and 27.9 in the CROSS and SELF treatments, respectively. The difference was not significant ($P = 0.92$ by unpaired Student's *t* test). The pollen tubes elongated in the exudate in random directions (Figs. 5 and 8). We could not find any evidence for a defect of elongation, such as a bulge or bursting at the tip of the pollen tubes. We also could not find strong fluorescence in pollen tubes that could be caused by cell death.

Some of the tubes were observed to elongate from the stigmatic exudate to the style hollow (Figs. 6 and 7) and form tight bundles (Fig. 6). The average ratio of pollen tubes in the style to pollen grains that germinated was 1.7–2.3 times higher in the CROSS treatments than in the SELF treatments in all three trees (Fig. 11). The difference was significant for Trees A and C and for the pooled data for all three trees. In most self-pollinated pistils, only a few pollen tubes entered the hollow, and the other pollen tubes continued to elongate in the stigmatic exudate (Figs. 9 and 10).

Once the pollen tubes started to elongate straightly in the styles, they did not seem to show defects in elongation. Because the pollen tubes elongated as a tight bundle, it was impossible to find the tip of each tube to measure pollen tube length. However, the proportions of styles in which the longest pollen tube(s) reached to the base of the style were almost the same in SELF (5/20) and CROSS (6/18) treatments, indicating that self-incompatibility is not caused by a defect in the elongation of the pollen tube in the style. Further, we did not find any evidence for elongation defects in the style, such as bulges at the tip or strong fluorescence in either CROSS or SELF treatments. In both treatments, pollen tubes periodically formed callose plugs (Fig. 6), indicating the good growth of pollen tubes.

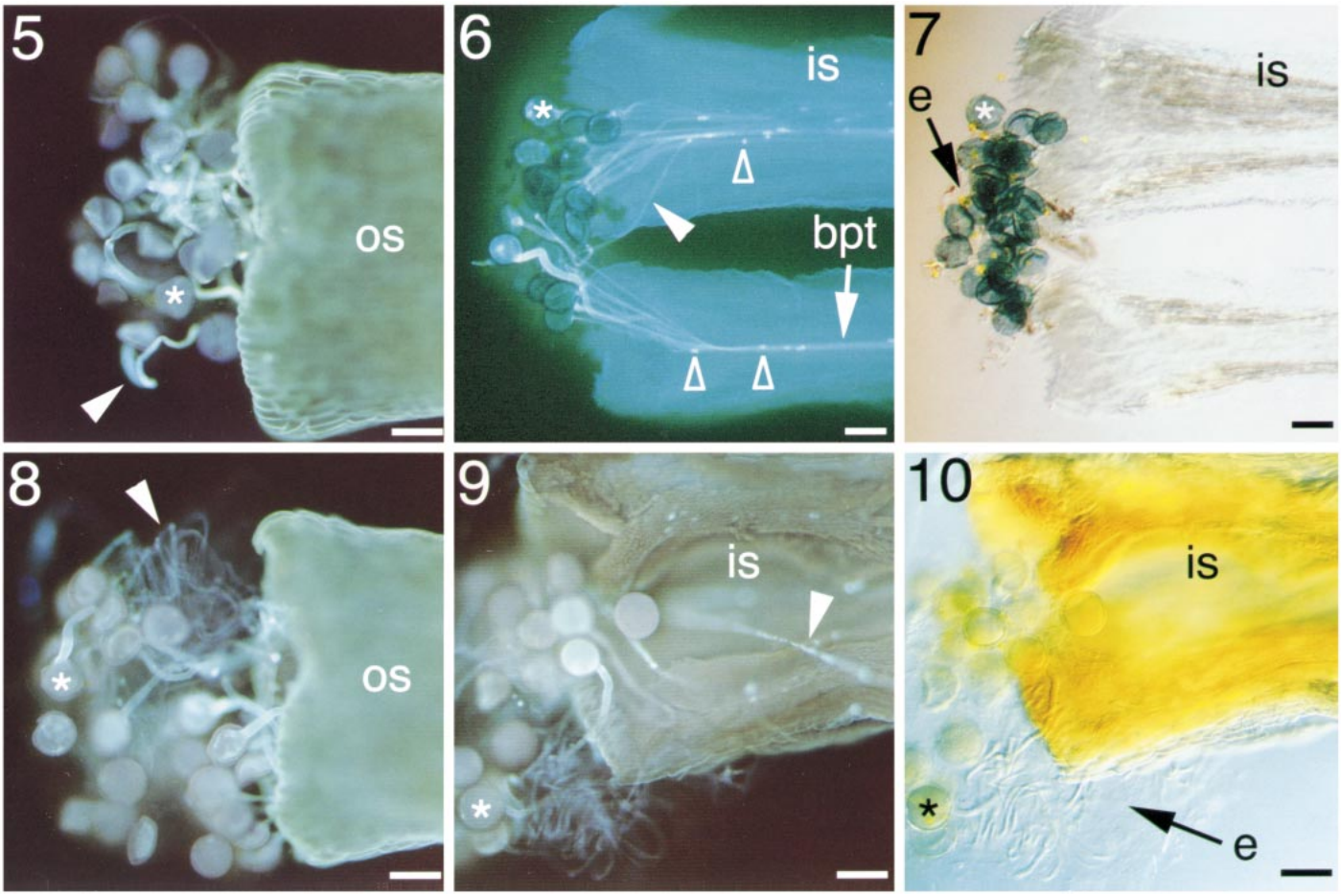
DISCUSSION

A pollen-tube guidance defect—The proportion of pollen tubes that entered the style was higher in the CROSS than in the SELF treatment (Figs. 5 and 6), indicating a partial self-incompatibility that inhibits self-pollen tubes from entering the style hollow. We found no evidence of incompatibility at the stage of pollen adhesion and germination in the stigmatic exudate, pollen tube elongation in the exudate, or pollen tube elongation in the style.

Early-acting self-incompatibility has typically been classified into two categories, depending on the reaction site of inhibition (de Nettancourt, 1977; Seavey and Bawa, 1986): inhibition of pollen germination and/or pollen tube elongation at the stigma (e.g., in *Brassica* [Takasaki et al., 2000]) and inhibition of pollen tube growth in the style (e.g., in Solanaceae [Newbigin, Anderson, and Clarke, 1993]). However, the incompatibility seen in *D. tempehes* differs from these types. The inhibition of self-pollen tubes occurred at the border of the stigma and the style hollow.

We suggest that this incompatibility reaction is not due to the absence of a component necessary for pollen germination or growth, nor to the existence of a substance interfering with the normal metabolism of the pollen grain or pollen tube, as has been previously suggested (de Nettancourt, 1977). Neither pollen germination nor pollen tube growth were inhibited or interfered with in SELF treatments in *D. tempehes*. Rather, it appeared that only the guidance of pollen tubes to the style hollow was inhibited. Similar guidance defects have been reported in previous mutant studies (Wilhelmi and Preuss, 1996; reviewed in Smyth, 1997) and in a study of interspecies incompatibility (Shimizu and Okada, 2000). Wilhelmi and Preuss (1996) studied a mutant of *Arabidopsis* and suggested the inhibition of adhesion between pollen tubes and pistil tissues. Shimizu and Okada (2000) studied the interspecies incompatibility in Brassicaceae and reported that there was a lack of directional guidance for pollen tubes to enter the ovules in ovaries. Either of these two models—a lack of adhesion substance or a lack of signal exchange for the directional guidance—can also explain the reaction of self-incompatibility in *D. tempehes*. The present results suggest that such a guidance defect contributes not only to interspecies incompatibility but also to intraspecies incompatibility. To our knowledge, *D. tempehes* is the first species in which a guidance defect is shown to cause self-incompatibility.

A late-acting mechanism—Most of the flower abortions occurred before ovary swelling. In that step, the difference in ovary survivorship was 10.0-fold between CROSS and SELF treatments using the pooled data for all three individuals (Table 1), which was much larger than the 2.2-fold difference caused



Figs. 5–10. Pollen tubes in CROSS and SELF treatments. Scale bars = 100 μm. Figs. 5–7 CROSS. Figs. 8–10 SELF. Figs. 5 and 8. Stigmatic region of a pistil. Pollen tubes wandered around. **6.** Another pistil dissected to show the interior. Pollen tubes (arrowhead) entered the hollow of the style, and formed into bundles (bpt). Callose plugs (open arrow) of pollen tubes can be seen. **7.** Bright field picture at the same focus as Fig. 6. **9.** The same pistil as in Fig. 8 was dissected. Only a few pollen tubes (arrowhead) were directed toward the style. **10.** Bright field picture at the same focus as Fig. 9. Figs. 7 and 10. The pollen grains and the pollen tubes were embedded in stigmatic exudate (e). Figs. 5, 6, 8, and 9 were observed with fluorescence. Figs. 7 and 10 were observed under a bright field. Abbreviations: * = pollen grains, arrowhead = pollen tube, open arrow = callose plugs, bpt = bundle of pollen tubes, e = stigmatic exudate, is = inner surface of the style, os = outer surface of the style.

by the pollen-tube guidance defect. Furthermore, we observed no inhibition of the elongation of self-pollen tubes in the style. There are two possible explanations for the discrepancy. The first is a threshold effect. Adhesion of a certain number of pollen grains is required for pollen germination in some species (reviewed by Hormaza and Herrero, 1994). In other plants, first pollen tubes ''pave the way'' for later arriving tubes. Both mechanisms are considered to be controlled by maternal substance. In *D. tempehes*, there was considerable variation among flowers in the proportion of pollen tubes entering styles (Fig. 11). The relationship between the number of pollen tubes in the style and probability of the fertilization is unknown in this species. It is possible that the numbers of pollen tubes entering the styles are below a required threshold in most of the self-pollinated ovaries. The second possible explanation for low ovary survivorship in the SELF treatment is late-acting self-incompatibility (Seavey and Bawa, 1986). Brewbaker (1957) suggested that ovarian incompatibility was often associated with the hollow styles that characterize *Dipterocarpus tempehes*. The uniform abortion that occurred before ovary swelling is also consistent with late-acting self-incompatibility (Seavey and Bawa, 1986). The relatively slow speed of fruit maturation before ovary swelling (Fig. 4) may be due to the incompatibility mechanism working before formation of a multicellular embryo.

Inbreeding depression and/or maternal selection—After ovary swelling, from stages II to IV, ovary survivorship was also higher in CROSS treatments (Table 1), although the difference between CROSS and SELF treatments was small compared to that between stages I and II. This selection probably occurred after fertilization and the formation of a multicellular embryo, since ovary swelling probably occurs only after fertilization in this species (ovaries >1 cm in diameter always had an embryo; T. Kenta, personal observation). Thus, we suggest the existence of either early-acting inbreeding depression (Wiens, 1984; Wiens et al., 1989) and/or late-acting maternal selection (Bookman, 1984; Stephenson and Winsor, 1986; Griffin, Moran and Fripp, 1987; Kenrick and Knox, 1989; Waser, 1993). Based on the present results, we cannot distinguish between growth failure by deleterious recessives in self-pollinated ovaries and preferential maternal resource allocation

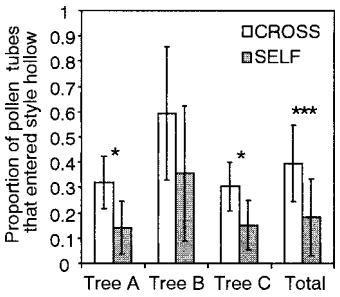


Fig. 11. The proportion of germinating pollen tubes that entered the style hollow in CROSS and SELF treatments. The difference between the treatments was tested by unpaired Student's *t* test (*$P < 0.05$, ***$P < 0.001$). Error bars indicate ± SD.

to cross-pollinated ovaries. In addition, a 1.6-fold difference in germination rate between cross and self-pollinated seeds suggests the possibility of inbreeding depression at that stage, although the difference was not statistically significant.

The breeding system—The result of much higher fruit sets (flower survivorship from stage I to IV) in the CROSS than in the SELF treatment (Table 1) indicates that *D. tempehes* has a strong self-rejection system. But this system was not perfect, because some fruit set was also observed in SELF. This result is consistent with the previous reports on other dipterocarps (Chan, 1981; Sakai et al., 1999a). The extremely low fruit set in the BAG treatment (Table 1) indicates the necessity of pollinators. In spite of the existence of effective pollinators, the result of higher fruit sets in CROSS than CONTROL proves that there was some degree of pollen limitation for fruiting under natural conditions. This pollen limitation caused a 2.8–5.1-fold difference in fruit set. Higher fruit set in CROSS might have been due to the exclusion of predators by the bag in CROSS treatment: in the CONTROL treatment, flowers were vulnerable to low survivorship due to predation. However, considering the low predation rate before fruit maturation (12.4%; T. Kenta, unpublished data), the difference in fruit set cannot be fully explained by predation exclusion.

In the self-rejection process of *D. tempehes*, we found early-acting self-incompatibility and inbreeding depression and/or maternal selection. Threshold effects in the number of pollen tubes for the fertilization or late-acting self-incompatibility were also implied. Thus we suggest that multiple processes determine the outcrossing rate of *D. tempehes*.

Dipterocarpus tempehes has relatively weak early-acting self-incompatibility caused by the unique pollen-tube guidance defect. On the other hand, many flowers were aborted at later stages. This seems to produce wastage of resource allocated to aborted flowers. Why, then, does *Dipterocarpus tempehes* not have strong self-incompatibility at an early stage? The flowering habit of *D. tempehes* and general flowering within the community are probably related to the advantages of this self-rejection system. In lowland dipterocarp forests, the interval between general flowering is highly variable (Ashton, Givinish, and Appanah, 1988; Appanah, 1993; Sakai et al., 1999b), and the synchrony of flowering within the population fluctuates between years. Pollinator population sizes also fluctuate due to fluctuation in the flowering of the plant community (Nagamitsu, 1998; Itioka et al., in press). On the other hand, *D. tempehes* flowers relatively frequently compared to the typical general flowering interval (T. Kenta et al., unpublished data). Thus, *D. tempehes* should experience variation in pollinator availability and pollination efficiency. Although the pollen limitation in *D. tempehes* detected in this study suggests that pollination efficiency is critical for this species, the strength of pollen limitation may change depending on the synchrony of flowering within the population and availability of the pollinator.

We suggest several adaptive significances of the weak early-acting self-incompatibility related with such an uncertain pollination efficiency. First, it would allow some fruiting when pollination efficiency is low. On the other hand, without some early-acting self-incompatibility, the flower abortion at later stage and resource wastage would increase. Second, if this species has a late-acting self-incompatibility mechanism, although this is not verified in this paper, a combination of the weak early-acting self-incompatibility and late-acting self-incompatibility would permit some flexibility in the choice of pollen parents. Seavey and Bawa (1986) pointed out that a late-acting self-incompatibility permits the maternal parent to evaluate the performance of pollen genotypes over a longer period and allows some flexibility in the choice of pollen parents in relation to resource availability, which is important in the face of variable pollen parentage and resource uncertainty. The same idea could be applied to species, like *D. tempehes*, with uncertain pollination efficiency. The partial early-acting self-incompatibility caused by the pollen guidance defect would preserve some flexibility in the choice of pollen parents and resource allocation.

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